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INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY (Chapter II of the Patent Cooperation Treaty) (PCT Article 36 and Rule 70)

Applicant's or agent's file reference 66146-48815		FOR FURTHER ACTION See Form PCT/IPEA/416																									
International application No. PCT/US04/18783	International filing date (day/month/year) 14 June 2004 (14.06.2004)		Priority date (day/month/year) 13 June 2003 (13.06.2003)																								
International Patent Classification (IPC) or national classification and IPC IPC(7): C12N 15/86 and US Cl.: 424/204.1																											
Applicant APATH, INC.																											
<p>1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.</p> <p>2. This REPORT consists of a total of 3 sheets, including this cover sheet.</p> <p>3. This report is also accompanied by ANNEXES, comprising:</p> <p>a. <input checked="" type="checkbox"/> (<i>sent to the applicant and to the International Bureau</i>) a total of 4 sheets, as follows:</p> <ul style="list-style-type: none"> <input type="checkbox"/> sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions). <input type="checkbox"/> sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box. <p>b. <input type="checkbox"/> (<i>sent to the International Bureau only</i>) a total of (indicate type and number of electronic carrier(s)) _____, containing a sequence listing and/or tables related thereto, in electronic form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).</p> <p>4. This report contains indications relating to the following items:</p> <table> <tbody> <tr> <td><input checked="" type="checkbox"/></td> <td>Box No. I</td> <td>Basis of the report</td> </tr> <tr> <td><input type="checkbox"/></td> <td>Box No. II</td> <td>Priority</td> </tr> <tr> <td><input type="checkbox"/></td> <td>Box No. III</td> <td>Non-establishment of opinion with regard to novelty, inventive step and industrial applicability</td> </tr> <tr> <td><input type="checkbox"/></td> <td>Box No. IV</td> <td>Lack of unity of invention</td> </tr> <tr> <td><input checked="" type="checkbox"/></td> <td>Box No. V</td> <td>Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement</td> </tr> <tr> <td><input type="checkbox"/></td> <td>Box No. VI</td> <td>Certain documents cited</td> </tr> <tr> <td><input type="checkbox"/></td> <td>Box No. VII</td> <td>Certain defects in the international application</td> </tr> <tr> <td><input type="checkbox"/></td> <td>Box No. VIII</td> <td>Certain observations on the international application</td> </tr> </tbody> </table>				<input checked="" type="checkbox"/>	Box No. I	Basis of the report	<input type="checkbox"/>	Box No. II	Priority	<input type="checkbox"/>	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability	<input type="checkbox"/>	Box No. IV	Lack of unity of invention	<input checked="" type="checkbox"/>	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement	<input type="checkbox"/>	Box No. VI	Certain documents cited	<input type="checkbox"/>	Box No. VII	Certain defects in the international application	<input type="checkbox"/>	Box No. VIII	Certain observations on the international application
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Date of submission of the demand 25 February 2005 (25.02.2005)	Date of completion of this report 28 November 2005 (28.11.2005)																										
Name and mailing address of the IPEA/US Mail Stop PCT, Attn: IPEA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (571) 273-3201	Authorized officer Vallorie Bell-Harres, Jr. Stacy B. Chen Telephone No. 571-272-1600																										

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Box No. I Basis of the report

1. With regard to the language, this report is based on:
 the international application in the language in which it was filed.
 a translation of the international application into English, which is the language of a translation furnished for the purposes of:
 international search (under Rules 12.3 and 23.1(b))
 publication of the international application (under Rule 12.4(a))
 international preliminary examination (under Rules 55.2(a) and/or 55.3(a))
2. With regard to the elements of the international application, this report is based on (*replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report*):
 the international application as originally filed/furnished
 the description:
pages 1-15 as originally filed/furnished
pages* NONE received by this Authority on _____
pages* NONE received by this Authority on _____
 the claims:
pages NONE as originally filed/furnished
pages* NONE as amended (together with any statement) under Article 19
pages* 1-19 received by this Authority on 25 February 2005
pages* NONE received by this Authority on _____
 the drawings:
pages 1-6 as originally filed/furnished
pages* NONE received by this Authority on _____
pages* NONE received by this Authority on _____
 a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing.

3. The amendments have resulted in the cancellation of:

- the description, pages _____
- the claims, Nos. _____
- the drawings, sheets/figs _____
- the sequence listing (*specify*): _____
- any table(s) related to the sequence listing (*specify*): _____

4. This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

- the description, pages _____
- the claims, Nos. _____
- the drawings, sheets/figs _____
- the sequence listing (*specify*): _____
- any table(s) related to the sequence listing (*specify*): _____

* If item 4 applies, some or all of those sheets may be marked "superseded."
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Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims <u>1-32</u>	YES
	Claims <u>NONE</u>	NO
Inventive Step (IS)	Claims <u>1-32</u>	YES
	Claims <u>NONE</u>	NO
Industrial Applicability (IA)	Claims <u>1-32</u>	YES
	Claims <u>NONE</u>	NO

2. Citations and Explanations (Rule 70.7)

Claims 1-32 meet the criteria set out in PCT Article 33(2)-(3), because the prior art does not teach or fairly suggest a synthetic, non-cytopathic negative-strand RNA virus replicon comprising a nucleotide sequence of said RNA virus, wherein the sequence of one or more structural genes is inactivated or deleted; and a nucleotide sequence encoding a selectable marker suitable for selection, wherein said sequence encoding a selectable marker is under the control of the RNA virus replication machinery and wherein the replicon, when introduced into a host cell, allows production of a stable culture of cells containing the replicon.

Applicant's arguments have been carefully considered.

Claims 1-32 meet the criteria set out in PCT Article 33(4), and thus have industrial applicability because the subject matter claimed can be made or used in industry.

NEW CITATIONS -----

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1. A synthetic, non-cytopathic negative-strand RNA virus replicon comprising
 - a) a nucleotide sequence of said RNA virus, wherein the sequence of one or more structural genes is inactivated or deleted; and
 - b) a nucleotide sequence encoding a selectable marker suitable for selection, wherein said sequence encoding a selectable marker is under the control of the RNA virus replication machinery and wherein the replicon, when introduced into a host cell, allows production of a stable culture of cells containing the replicon.
 2. The replicon of claim 1, wherein said sequence encoding a selectable marker is a gene that confers resistance to an antibiotic.
 3. The replicon of claim 2 wherein said gene is a *bsd* gene.
 4. The replicon of claim 1, further comprising a sequence encoding a heterologous protein.
 5. The replicon of claim 1 further comprising a reporter gene.
 6. The replicon of claim 5, wherein said reporter gene is a gene encoding green fluorescent protein (GFP).
 7. The replicon of claim 1 wherein said RNA virus is respiratory syncytial virus (RSV).
 8. The replicon of claim 7, wherein the sequence encoding the F, G and SH glycoproteins is deleted.
 9. The replicon of claim 8 wherein said sequence encoding a selectable marker is a gene that confers resistance to an antibiotic.
 10. The replicon of claim 9, wherein said gene is a *bsd* gene.
 11. The replicon of claim 10, further comprising a reporter gene.

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12. The replicon of claim 11 wherein said reporter gene is a gene encoding GFP.
13. A cell line comprising the replicon of claim 1.
14. The replicon of claim 12, wherein said replicon is harbored in a cell line selected from the group consisting of BHK-RR-B51E (ATCC deposit number PTA-5257) and HeLa-RR-B51S (ATCC deposit number PTA-5258).
15. The replicon of claim 12, further comprising a sequence encoding a heterologous protein.
16. A cDNA of a non-cytopathic negative-strand RNA virus replicon comprising
 - a) a nucleotide sequence complementary to the genome of said RNA virus, wherein the sequence encoding one or more structural genes is inactivated or deleted;
 - b) a nucleotide sequence comprising a heterologous promoter sequence operatively linked to said sequence of a); and
 - c) a nucleotide sequence comprising a gene encoding a selectable marker suitable for selection, wherein said gene is under the control of the RNA virus replication machinery and wherein the cDNA, when introduced into a host cell, allows production of a stable culture of cells containing the replicon.
17. The cDNA of claim 16, wherein said heterologous promoter sequence is selected from the group consisting of T7 polymerase promoter, cytomegalovirus immediate early promoter, SV40 early promoter and polymerase I promoter.

18. The cDNA of claim 16 wherein said promoter is a T7 polymerase promoter.
19. A replicon encoded by the cDNA of claim 16.
20. A method comprising
 - a) transfecting a cell line in culture with a polynucleotide comprising

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i) a DNA sequence complementary to a negative-strand RNA virus replicon, wherein the sequence encoding one or more structural proteins is inactivated or deleted;

ii) a DNA sequence comprising a gene encoding a selectable marker protein suitable for selection;

b) culturing said cell line *in vitro*;

c) selecting for cell populations displaying the phenotype conferred by said selectable marker, thereby producing a stable culture of cells containing the negative-strand RNA virus replicon; and

d) isolating RNA virus sequences from said cell populations of c).

21. The method of claim 20, wherein said selectable marker is a gene that confers resistance to an antibiotic.

22. The method of claim 21, wherein said antibiotic is blasticidin.

23. The method of claim 21, wherein said selecting of c) comprises culturing said cell line in a medium containing an antibiotic.

24. The method of claim 20, wherein said RNA virus is RSV.

25. The method of claim 24, wherein said RSV sequence comprises a mutation or deletion that renders the F, G and SH glycoproteins inoperative.

26. A method comprising

a) transfecting a cell line in culture with

i) a DNA sequence complementary to a negative-strand RNA virus replicon, wherein the sequence encoding one or more glycoproteins is inactivated or deleted and wherein said sequence comprises a T7 polymerase promoter operatively linked to said sequence of i), and wherein said sequence further encodes a selectable marker; and

ii) a DNA sequence encoding a T7 polymerase;

b) culturing said cell line *in vitro*;

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c) selecting for cell populations displaying the phenotype conferred by said selectable marker thereby producing a stable culture of cells containing the negative-strand RNA virus replicon; and

d) isolating RNA virus sequences from said populations of c).

27. The method of claim 26, wherein step a) further comprises transfecting said cell line with support plasmids encoding viral proteins necessary for replication and mRNA synthesis.

28. A method for mobilizing a negative-strand RNA virus replicon comprising

- a) transfecting the cell line of claim 13 with a plasmid encoding a viral glycoprotein that allows virion formation;
- b) culturing said cell line of a) in culture medium;
- c) inoculating a fresh cell line with virions present in the culture medium of b).

29. The method of claim 28 wherein said viral glycoprotein that allows virion formation is a VSV G protein.

30. The method of claim 28, wherein said selectable marker is a gene that confers resistance to an antibiotic, said method further comprising

- d) culturing said inoculated cells of c) on medium containing the antibiotic; and
- e) identifying replicon-expressing cells from the surviving cells.

31. A method comprising culturing a cell line containing the replicon of claim 4 in vitro to produce said heterologous protein.

32. A method for screening for antiviral agents comprising

- a) contacting the cell line of claim 13 with a candidate agent, and
- b) testing for an increase or decrease in replication or activity of the RNA virus replicon relative to a control cell line harboring the same replicon, but which control cell line has not been contacted with the candidate agent.